(51) International Patent Classification 6 :		(11) International Publication Number: WO 96/15813
A61K 48/00, C12N 15/00, 5/00, 5/06	A1	(43) International Publication Date: 30 May 1996 (30.05.96)

(54) Title: CELL COMPOSITIONS FOR USE IN TRANSPLANTATION AND EX VIVO GENE THERAPY

(57) Abstract

Methods for the preparation of bone marrow stem cell compositions for use in transplantation therapy and ex vivo gene therapy of a mammal. The methods involve the use of quiescent stem cells for transplantation therapy and quiescent transfected stem cells for ex vivo gene therapy.

CELL COMPOSITIONS FOR USE IN TRANSPLANTATION AND *EX VIVO* GENE THERAPY

Cross-Reference to Related Applications

5   This application is a continuation-in-part of earlier filed U.S. application Serial No. 08/344,080, filed November 23, 1994, which application is incorporated herein by reference in its entirety and is the basis of a claim for priority under 35 U.S.C. § 120.

Statement as to Federally Sponsored Research

10   This invention was made at least in part with funds from the Federal government, and the government therefore has rights in the invention.

Background of the Invention

15   The invention relates to the field of stem cell engraftment and human *ex vivo* gene therapy.

Bone marrow transplantation is often accompanied by marrow cytotoxic therapy to create marrow space or niches. The usual method of creating such niches is by

20 irradiation and/or chemotherapy treatment. This practice has been challenged by Brecher, Saxe and colleagues, who demonstrated that bone marrow cells (e.g., hematopoietic stem cells) engraft into normal non-myeloablated hosts (Brecher, G. et al. (1982) PNAS USA 79:5085; and Saxe,

25 D.F. et al. (1984) Exp. Hematol. 12:277). These investigators showed varying levels of engraftment up to 25% after infusion of normal bone marrow into non-myeloablated hosts.

One theoretical approach to transplantation

30 involves procurement of stem cells for transplantation followed by expansion of the stem cells to increase their number prior to infusion into the host. Cellular expansion and/or induction into active cell cycling is

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accomplished by contacting cytokines or other agents,
such as 5-fluorouracil, with the stem cells *in vivo*, if
expansion occurs prior to collection of stem cells; or *in
vitro*, if expansion occurs following collection of stem
5   cells.   Long-term repopulation of bone marrow with
cytokine-treated cells has been assessed *in vivo* (Bodine
D.M., Blood, 79:913, 1992; Szilvassy S.J. and Cory, S.,
Blood 84:74, 1994; Li, C.L. and Johnson, G.R. Blood
84:408, 1994; and Ramshaw, H.S. et al., Experimental
10 Hematology, 22:823, No. 548, 1994).

        Additionally, cytokines have been used in various
combinations to enhance retroviral integration into stem
cells by increasing the percent of stem cells in the
process of active cell cycle.   *In vitro* cytokine
15 incubations have been used to induce active cell cycling
in hematopoietic cells to facilitate retroviral
integration in various gene therapy approaches (Bodine
D.M. et al., Exp Hematol 19:206, 1991; Szilvassy S.J. and
Cory, S., Blood 84:74, 1994; and Kittler, E.L.W. et al.,
20 Blood 84:344, 1994 (abstr. suppl I)).   Subsequent to
retroviral integration, these actively cycling
transfected stem cells are introduced into a mammal so
that they engraft in the bone marrow of the mammal.   Thus
the success of *ex vivo* gene therapy is dependent not only
25 on the efficiency of retroviral integration, but also on
the efficiency of transfected stem cell engraftment.

        There is a great need for methodologies to enhance
engraftment of stem cells in a host mammal for the
purpose of improved human bone marrow transplantation
30 therapy as well as for improved human *ex vivo* gene
therapy.

Summary of the Invention

        Applicant discloses that rendering cells
qui scent prior to transplantation results in improved

long term engraftment, i.e., for time periods greater
than approximately 6 weeks post-transplantation.
Accordingly, the invention features stem cell
compositions and methods of preparing stem cell
5 compositions for transplantation or *ex vivo* gene therapy
that significantly enhance engraftment of the
transplanted stem cells, wherein the stem cells are
expanded to produce a population of expanded stem cells.
The expanded stem cells are next treated so that a
10 majority of them become quiescent stem cells to form the
stem cell composition that is introduced into a mammal by
standard stem cell transplantation techniques.   In
preferred embodiments the mammal is a non-myeloablated or
a myeloablated host mammal, preferably a human patient.

15   Stem cells are expanded and/or treated to enter
active cell cycling *in vivo* or *in vitro* by methods well
known to those skilled in the art and include, but are
not limited to, the administration of a cytokine, e.g.,
stem cell factor (Steel factor), IL-3-CHO, IL-6, IL-11,
20 and the like, to the stem cell *in vivo* or *in vitro*; and
administration of 5-fluorouracil (5-FU) *in vivo*.

       Expanded and/or actively cycling stem cells are
treated to become quiescent according to the invention by
methods well known to those of ordinary skill in the
25 relevant arts and include, but are not limited to, serum
starvation and removal of the expanding or cell cycling-
inducing agent.   Cells are judged to be quiescent when
fewer than 30%, preferably fewer than 10%, more
preferably fewer than 5% of the cells are judged to be
30 progressing through the cell cycle as determined by the
high specificity tritiated thymidine [^{3}HtdR] suicide
technique which selects only fully proliferating cells,
i.e., replicating cells.   Quiescent cells can also be
observed by the lack of incorporation of a radio-labelled
35 nucleotide into the DNA of a repr sentative portion of

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the cell population of interest. Quiescent cells are
also observed as exhibiting a single DNA peak as measured
by flow-cytometry (a single peak indicates that the cells
are quiescent, while more than one peak indicates DNA
5 synthesis).

The invention also features stem cell compositions
and methods of preparing stem cell compositions for use
in *ex vivo* gene therapy in which the stem cells are
induced to proliferate to enhance retroviral vector
10 integration, and then induced to become quiescent prior
to introduction into a mammal. The invention also
features the gene therapy method. In preferred
embodiments, the therapeutic method is used for treating
an inherited, an acquired, or a metabolic deficiency in a
15 mammal (such as a human). For example, the transfected
stem cells may contain expressible DNA for the production
of antisense RNA to reduce the expression of an
endogenous gene of the mammal. In other preferred
embodiments, the transfected stem cell may contain DNA
20 encoding a protein capable of preventing or treating an
inherited or acquired disease, e.g., Factor VIII
deficiency in hemophilia, cystic fibrosis, and adenosine
deaminase deficiency.

Infused cells or their progeny preferably contain
25 a marker such that the infused cells can be observed in a
population of host cells for the purpose of measuring the
level of engraftment.

Another embodiment of the invention is a method of
transplanting a predetermined number of quiescent stem
30 cells into a non-myeloablated host mammal in which a
first fraction of the stem cells is introduced into the
host mammal at a first point in time followed by
introducing thereafter the remaining cells in at least 5,
and not gr ater than 15, additional administrations.
35 Preferably each administration is spaced from the other

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by at 1 ast 12 hours. At a final point in time a final
fraction of the predetermined number of cells is
introduced such that all of the cells have been
introduced into the host mammal.

5   The invention also features a method of *ex vivo*
gene therapy in which transfected pluripotent
hematopoietic stem cells are treated to become quiescent
transfected stem cells. A predetermined number of the
transfected stem cells are introduced into the host
10 mammal such that a first fraction of the cells are
introduced at a first point in time followed by
introducing thereafter the remaining cells in at least 5,
and not greater than 15, additional administrations, each
administration being spaced from the other by at least 12
15 hours. At a final point in time, a final fraction of the
predetermined number of transfected quiescent stem cells
is introduced such that all of the original predetermined
number of cells have been introduced into the host
mammal.

20   In a preferred embodiment of the scheduled
transplantation for enhancement of engraftment of
quiescent stem cells or quiescent transfected stem cells,
the scheduled transplantation involves a predetermined
number of cells wherein the number is at least 1×10^8
25 cell/kg body weight of the host mammal and not more than
8×10^9 cell/kg body weight of the host mammal.

In another preferred embodiment of the scheduled
transplantation methods, the fractions of the
predetermined stem cells are substantially equal in
30 number of cells. Preferably, the introduction of the
quiescent stem cells, or quiescent transfected stem
cells, occurs at least 5, and not more than 15, days
after the first introduction of a fraction of the stem
cells. Preferably, th introductions of said cells are

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spaced approximately equally in time from the first
introduction to the final introduction of the cells.

In another embodiment of the invention, a method
of providing short term and long term bone marrow
5   transplantation is provided. A population of expanded
stem cells is divided into first and second fractions.
The first fraction is used to prepare a first cell
compositions, and is introduced into a host mammal by a
single administration, or preferably by multiple,
10   scheduled administrations. The second fraction is
induced to become quiescent prior to introduction into
the host mammal, and is used as the second cell
composition, as a single administration or preferably by
the scheduled administration of multiple fractions.

15   In another embodiment of the invention, a method
of providing short term and long term *ex vivo* gene
therapy is provided. A population of expanded
transfected stem cells is divided into first and second
fractions. The first fraction is introduced into a host
20   mammal by a single administration of cells or preferably
by the scheduled administration of multiple fractions.
The second fraction of expanded transfected stem cells is
induced to become quiescent prior to introduction into
the host mammal as a single administration or preferably
25   by the scheduled administration of multiple fractions.

In yet another embodiment of the invention, a
method of providing short term and long term bone marrow
transplantation is provided in which a population of
expanded stem cells is separated into fractions, a first
30   fraction being enriched in short term repopulating stem
cells, and a second fraction being enriched in long term
repopulating stem cells. The first fraction of expanded,
actively cycling stem cells is introduced into a host
mammal for short term engraftment (engraftment up to
35   approximately 6 w eks post-transplantation). The s cond

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fraction of expanded, actively cycling stem cells is
induced to become quiescent prior to introduction into a
host mammal, thus allowing long term engraftment
(engraftment after approximately 6 weeks post-
5   transplantation).

        In another embodiment of the invention, an *ex vivo*
gene therapy method is provided in which a population of
expanded stem cells is separated into fractions, a first
fraction being enriched in short term repopulating stem
10 cells, and a second fraction being enriched in long term
repopulating stem cells. According to the method,
transfection of the expanded stem cells, e.g., by
retroviral transfection, with exogenous DNA is performed
prior to separation of the stem cells into fractions
15 enriched with early or late repopulating stem cells.
According to the method, transfection is optionally
performed after separation of the stem cells into
fractions. The first fraction of transfected expanded,
actively cycling stem cells is introduced into a host
20 mammal for short term engraftment and short term *ex vivo*
gene therapy. The second fraction of transfected
expanded, actively cycling stem cells is induced to
become quiescent prior to introduction into a host
mammal, thus allowing long term engraftment and long term
25 *ex vivo* gene therapy.

        By the term "stem cell" or "hematopoietic stem
cell" is meant a pluripotent cell of the hematopoietic
system capable of differentiating into cells of the
lymphoid and myeloid lineages.
30   By the term "transfected stem cell" is meant a
stem cell into which exogenous DNA or an exogenous DNA
gene has been introduced by retroviral infection or other
means well known to those of ordinary skill in the art.
        By the term "*ex vivo* gene therapy" is meant the *in*
35 *vitro* transfection or retroviral infection of stem cells

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to form transfected stem cells prior t introducing the
transfected stem cells into a mammal.

By the term "quiescent stem cell" is meant a stem
cell in the G_1 or G_0 phase of the cell cycle. A
population of cells is considered herein to be a
population of quiescent cells when at least 50%,
preferably at least 70%, more preferably at least 80% of
the cells are in the G_1 or G_0 phase of the cell cycle.
Quiescent cells exhibit a single DNA peak by flow-
cytometry analysis, a standard technique well known to
those of ordinary skill in the arts of immunology and
cell biology. Another technique useful for determining
whether a population of cells is quiescent is the
addition of a chemical agent to the cell culture medium
that is toxic only to actively cycling cells, i.e., DNA
synthesizing cells, and does not kill quiescent cells.
Non-exclusive examples of such chemical agents include
hydroxyurea and high specific activity tritiated
thymidine (^{3}HtdR). A population of cells is evaluated as
to the percent in an actively cycling state by the
percent of the cell population killed by the chemical
agent. A cell population in which *in vitro* tritiated
thymidine killing is less than approximately 30%,
preferably less than approximately 10%, more preferably
less than approximately 5%, is considered to be
quiescent.

By the term "early repopulating stem cells" is
meant stem cells which are capable of engrafting into the
bone marrow of a host mammal within approximately 6 weeks
post-transplantation.

By the term "late repopulating stem cells," also
termed "long-term repopulating cells" is meant
myelolymphoid stem cells which are capable of engrafting
into the bone marrow of a host mammal after approximately
6 weeks post-transplantation.

By the term "engrafting" or engraftment" is meant
the persistence of proliferating stem cells in a
particular location over time. Thus, early repopulating
stem cells do not persist for more than about 6 weeks,
5   whereas late repopulating stem cells persist for longer,
and preferably much longer, than about 6 weeks.

Cycling stem cells can be treated to become
quiescent by serum or isoleucine starvation. Quiescence
can also be induced by reduction of nutrients in the
10 culture medium such that the cycling stem cells enter and
remain in the G_1 or G_0 phase of the cell cycle while the
nutrient level is reduced. These methods can be used
alone or in combination.

By the term "expanded population" is meant a
15 population of cells, wherein at least 50% of the cells
have divided at least once. In certain embodiments of
the invention, the cells may be induced to divide by the
administration of cell cycling agents such as 5-FU and/or
cytokines such as IL-3-CHO, IL-6, IL-11, and other growth
20 stimulating factors well known to those of ordinary skill
in the art of immunology.

By the term "non-myeloablated host mammal" is
meant a mammal which has not undergone irradiation, or
other treatment (such as chemical treatment) well known
25 to those of ordinary skill in the art, to cause the death
of the bone marrow cells of the mammal.

By the term "myeloablated host mammal" is meant a
mammal which has undergone irradiation, or other
treatment, such as chemical treatment, well known to
30 those of ordinary skill in the art, to cause the death of
at least 50% of the bone marrow cells of the mammal.

The invention provides methods for the treatment
of myelolymphoid cell disorders and stem cell
compositions for use in the treatments. Th invention
35 affords unique and critical advantag s over previous

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methods. Specifically, the invention provides for
increased engraftment of transplanted cells by
transplantation of quiescent stem cells or quiescent
transfected stem cells into a myeloablated or a non-
5 myeloablated host mammal. Enhanced stem cell engraftment
is useful for treating diseases caused by impaired
function of bone marrow cells. Examples of such diseases
include, but are not limited to, lymphoma, multiple
myeloma, breast cancer, testicular cancer, leukemias,
10 congenial hemolytic anemias (sickle cell anemia and
thalassanemia) and some immunodeficiency diseases, e.g.,
acute leukemia, Hodgkin's and non-Hodgkin's lymphoma, and
neuroblastoma. *Ex vivo* gene therapy is also enhanced by
the improved engraftment of transfected stem cells
15 according to the method of the invention. Such
transplantation of quiescent stem cells and the resultant
increased engraftment permits amplification of a desired
therapeutic effect.

    The improved engraftment achieved using the
20 methods of the invention is also useful in high-dose
chemotherapy regimens. The hematologic toxicity observed
with multiple cycles of high-dose chemotherapy is
relieved by conjunctive administration of autologous
hematopoietic stem-cells. Diseases for which reinfusion
25 of stem cells (cells not induced to be quiescent) has
been described include acute leukemia, Hodgkin's and non-
Hodgkin's lymphoma, neuroblastoma, testicular cancer,
breast cancer, multiple myeloma, thalassemia, and sickle
cell anemia (Cheson B.D., et al. (1989) Ann Intern Med.
30 **110**:51-65; Wheeler, C. et al. (1990) J. Clin. Oncol.
8:648-656; Takvorian, T. et al. (1987) N. Engl. J. Med.
316:1499-1505; Yeager, A.M. et al. (1986) N. Eng. J. Med.
315:141-147; Biron, P. et al. (1985) in <u>Autologous Bone
Marrow Transplantation: Proce dings of th First</u>
35 <u>International Symposium</u>, Dick , K.A. et al., eds, p. 203;

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Peters, W.P. (1985) ABMT, *supra*, p. 189; Barlogie, B. (1993) Leukemia 7:1095; Sullivan, K.M. (1993) Leukemia 7:1098-1099). Treatment of such diseases can be improved by the method of the present invention of administering
5   cells known to be quiescent and therefore capable of engrafting at an increased level in a host mammal which has or has not been subjected to myeloablation.

Another advantage of the invention is the reduced risk to the recipient of the transplanted cells since no
10 myeloablation is necessary to achieve superior short and long-term engraftment in the non-myeloablated host mammal.

Another advantage of the invention is that under conditions in which the host is myeloablated, the method
15 of the invention provides superior long term engraftment by administering cells known to be quiescent.

Another advantage of the invention is the ability to use bone marrow, mobilized peripheral blood, and cord blood stem cells in clinical transplant therapy or *ex*
20 *vivo* gene therapy.

An advantage of the present invention is its ability to provide useful methods for increased engraftment of stem cells or transfected stem cells by controlling the cell cycle state of the transplanted
25 cells.

Other features and advantages of the invention will be apparent from the following detailed description thereof, and from the claims.

Brief Description of the Drawings
30   Figs. 1A and 1B are graphs of the results of a study on the influence of preincubation of donor bone marrow cells in growth medium containing the cytokines IL-3, IL-6, IL-11, and SCF (stem c 11 factor).

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Fig. 2 is a bar graph showing the results of studies in which male BALB/c mouse bone marrow was incubated in culture media alone or in media plus cytokines.

5   Figs. 3A and 3B are bar graphs representing progenitor cell expansion of whole marrow in culture medium with the cytokines mSCF (mouse stem cell factor), IL-3, IL-6, and IL-11 for 48 hours for cell types Ly5.2 (3A) and Ly5.1 (3B), which cells differ in a single

10   surface marker allowing engraftment of the different populations of cells to be monitored by anti-Ly5.2 antibody staining.

Figs. 4A and 4B are three-dimensional bar graphs of a cell-cycle analysis, using a thymidine suicide

15   assay, of noncultured cells (marrow) and cytokine-treated cells (progenitor cells) incubated for 48 hours in culture medium with the cytokines mSCF, IL-3, IL-6, and IL-11. The results for Ly5.2 cells are shown in Fig. 4A and the results for Ly5.1 cells are shown in Fig. 4B.

20   Figs. 5A to 5C are graphs of bone marrow engraftment after competitive transplantation of noncultured cells with cytokine-treated cells. FACS (Fluorescent Activated Cell Sorting) analysis of donor-derived cells stained with monoclonal antibodies specific

25   for Ly5.2 was performed at 12 weeks (5A and B) and 22 weeks (5C). The percent engraftment as a function of culturing or non-culturing is presented.

Detailed Description

Methods for the enhancement of bone marrow stem

30   cell engraftment are provided for use in transplantation therapy and ex vivo gene therapy of a mammal. The methods involve the induction of cellular expansion of the stem cells and the induction of the cells to become

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quiescent prior to transplantation of the stem c lls for
transplantation therapy and for *ex vivo* gene therapy.

Short-term *in vitro* incubation of murine or human
marrow cells with a variety of single cytokines or growth
5   factor combinations results in expansion of hematopoietic
progenitor cells (HPC) as assayed in *in vitro* colony
systems including multipotent colony-forming cells, long
term culture-initiating cells (LTC-IC), and low- and
high-proliferative potential colony-forming cells (LPP-
10 and HPP-CFCs).

In one study detailed below, *in vitro* cytokine-
treated cells, as opposed to cells harvested after *in
vivo* chemotherapy, were administered in competition with
untreated cells and analyzed for their contribution to
15 engraftment. Primitive hematopoietic cells induced to
divide and proliferate by cytokines in culture are
inhibited in the ability to engraft (possess an
engraftment defect) prior to approximately 6 weeks post-
transplantation. The defect is related to cell cycle
20 activation of the donor cells. Competitive transplant
studies in nonlymphomyeloablated hosts demonstrated
marrow cells harvested 6 days after *in vivo* treatment of
mice with 5-FU exhibit an engraftment defect and that the
defect is no longer present if the cells are harvested 35
25 days after treatment with 5-FU. Using the same animal
model, a cytokine-induced engraftment defect was shown.
In both cases, the engraftment defect correlated with
cell-cycle activation.

Protocols designed to promote mitosis of
30 hematopoietic progenitors for retroviral gene transfer
frequently contain cytokine combinations including SCF,
IL-3, IL-6, and IL-11 rendering them unlikely to engraft.
Enhancing engraftment efficacy of cytokine-treated stem
cells by reversing cell-cycle-relat d defects are useful

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both to improve the clinical potential for cytokine-
expanded stem cells and for gene therapy approaches.

In Examples 7 and 8, a report is provided of two
competitive studies in which cytokine-treated cells,
5 which were not pretreated *in vivo* to proliferate (e.g.,
day 2 post 5-FU), competed with noncultured fresh marrow
cells in lethally irradiated recipients for engraftment.
The effects on engraftment of *in vitro* culture of marrow
cells with interleukin-3 (IL-3), IL-6, IL-11, and stem
10 cell factor (SCF) on progenitor cell expansion, cell-
cycle status, and competitive repopulation *in vivo* was
examined.

Two competitive murine transplant models using
irradiated host mice were employed to examine the
15 engraftment potential of marrow cells cultured for 48
hours with the cytokines mSCF, IL-3, IL-6, and IL-11.
The first model, which uses the Ly-5.1/Ly-5.2 congenic
B6.SJL and C57BL/6 strains, allows distinction of donor-
and host-derived cells with monoclonal antibodies
20 specific for the alleles of Ly-5; the second model uses
BALB/c mice, allowing for the detection of male donor
cell DNA sequences in female recipients.

General Procedures

Animals

25   Male and female BALB/c mice were obtained from the
Jackson Laboratory (Bar Harbor, ME). C57BL/6 (Ly5.2) and
B6.SJL-Ly5a (Ly5.1) mice were purchased from The Jackson
Laboratories (Bar Harbor, ME), and BALB/c mice were
purchased from Charles River Laboratories (Wilmington,
30 MA). All animals were maintained in virus-free
conditions and were given food and acidified water ad
libitum. Mice were purchased at either 16 to 18 g or 18
to 22 g and were housed for minimum of 1 week before
experimental use.

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Tritiated Thymidine Suicide Assay

A standard *in vitro* [3]HTdR suicide technique
(Iscove, N.N. et al. (1970) Proc. Soc. Exp. Med. 134:33;
and Quesenberry, P.J. and Stanley, K. (1980) Blood
5 56:1000), well known in the art, was performed as
follows. A population of 5×10^6 marrow cells per ml
were incubated at 37°C for 20 minutes in Hanks' balanced
salt solution (HBSS) containing 200 μCi per ml ^{3}H-TdR
(New England Nuclear: specific activity = 20.0 Ci/mmol/L)
10 or a concentration of nonradioactively labelled thymidine
equivalent to that in the [3]HTdR incubation. After
incubation, the cell suspensions were washed three times
and then plated at an equivalent cell density of 25,000
per dish based on the original counts for progenitor cell
15 growth *in vitro* in soft agar as described above. The
fraction of cells actively cycling was determined by
percent reduction of HPP-CFC induced by [3]HTdR exposure.

Soft-agar Colony-forming Assay

To evaluate the colony forming ability of test
20 cells, *in vitro* agar colony-forming assays were performed
as follows. Both freshly procured uncultured cells and
cells obtained after 48 hours in culture were counted and
placed at a cell density of 25,000 per 35-mm plastic
petri dish in a double-layer nutrient agar system.
25 Underlays were α-MEM with 15% FCS supplemented with
vitamins, glutamine, and growth factors (colony-
stimulating factor [CSF-1], granulocyte-macrophage-CSF
[GM-CSF], G-CSF, IL-1α, IL-3, mSCF, and bFGF). Target
cells were included in a 0.5-ml overlay with a final agar
30 concentration of 0.33%. Dishes were gassed with a
mixture of 5% O_2, 10% CO_2, and 85% N_3, and incubated at
37°C for 14 days. Four dishes were prepared from each
animal. Dishes were scored on a dissecting microscope
for LPP-CFC (all cell clusters with more than 50 cells
35 and not fulfilling HPP-CFC criteria) and HPP-CFC (highly

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dense colonies great r than 0.5 mm in diameter or
moderately dense colonies greater than 1.0 mm in
diameter) (Peters, S.O. et al., (1995) Exp. Hematol.
23:461).

Stem Cell Sorting

A non-limiting method of sorting a population of
stem cells into populations enriched in either early or
late repopulating stem cells is provided below. The
method utilizes differential staining characteristics of
stem cells to allow fluorescence-activated cell sorting
(FACS). It is understood the appropriate antibodies are
chosen by one of ordinary skill in the art to be
appropriate for the cell strain being manipulated.

In an exemplary method for isolating late
repopulating stem cells (identified as Hoechst low,
rhodamine low, cells by FACS analysis), cells are
prepared for FACS by obtaining the cells from the femurs
and tibiae of mice (e.g., C57BL/6 mice, or the like) by
flushing using a 2.5 ml syringe with a 21-gauge needle.
After washing, the bone marrow cells are resuspended in
PBS/2%BSA (phosphate buffered saline/2% bovine serum
albumin) and passed through a 40 μm filter (Falcon #2340,
VWR #21008-949, for example). Filtered cells are diluted
to 10^7 cells/ml with PBS and centrifuged for 20 minutes,
1000 x g (1800 rmp) 4°C. Cells are collected and washed
with PBS/2%BSA and centrifuged at 1500 rmp, 10 minutes at
4°C. Collected cells are resuspended to 10^8 cells/ml in
PBS/2%BSA and incubated for 15 minutes at 4°C with an
equal volume of antibody mixture: Ter 119, 0.1 μg/ml;
B220 (15 μl/10^8 cells, Nature, 289:681 (1981); MAC-1 (15
μl/10^8 cells); GR-1 15 μl/10^8 cells; 7/4 (400 μl/1.5 x 10^8
cells, Immunogenetics, 18:229 (1983)); Lyt-2 (1/20 final
dilution, BD/Collaborative - lot 903514); L3T4 (1/20
final dilution, BD/Collaborative - lot 903554); YW25.12.7

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(1/10 final concentration, Mol. Cell Probes, $\underline{1}$:297 (1987)).

     Following incubation with the antibodies, the cells are diluted to 50 ml with PBS/2%BSA and centrifuged
5 at 1500 rpm for 10 minutes. Cells are collected and the cell suspension volume is adjusted to 2 ml with PBS/2%BSA. To the cells is added a 2 ml suspension of washed DYNALBEADS450® (Dynal Inc., Oslo, Norway) dropwise with swirling. The cell/bead mixture is centrifuged for
10 3 minutes at 40 rpm and the cells are resuspended by pipetting. Suspension volume is adjusted to 7 ml and the beads together with the cells bound to them are removed magnetically. The mixture is washed with 40 ml PBS/2%BSA and centrifuged for 10 minutes at 1500 rpm at 4°C.
15   Cells are collected and resuspended to 10^6 cells/ml, rhodamine is added to 0.1 μg/ml final concentration and incubated 20 minutes at 37°C in the dark. The cells are centrifuged 10 minutes at 1500 rpm, and the cells are resuspended to 10^6 cells/ml with 10 μM
20 Hoechst and incubated for 1 hour at 37°C. Cells are washed in cold PBS/2%BSA, centrifuged, collected, and resuspended to approximately 1-5 x 10^6 cells/ml. Cells are evaluated on a FACS instrument using Blast window, 3% Hoechst, and 10% rhodamine.
25   In an exemplary procedure for collecting separate populations of cells enriched in early or late repopulating stem cells the following conditions can be used (Li, C.L. and Johnson, G.R. Blood $\underline{84}$:408 (1994)), with the antibodies chosen for the specific cell mixture
30 being analyzed. Cells are prepared for FACS by obtaining the cells from the femurs and tibiae of mice as described above. After washing, the bone marrow cells are resuspended in 10 ml balanced salt solution containing 5% fetal calf serum (BSS.FCS) and 0.1 μg/ml rhodamine 123

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(Eastman Kodak Co., Rochester, NY), and incubated at 37°C
for 30 minutes.

        After washing once, the cells are resuspended in
10 ml fresh BSS.FCS and incubated at 37°C for 30 minutes.
5   The cell suspension is then diluted further by the
addition of 10 ml BSS.FCS, layered on top of FICOLL®
(Pharmacia Inc. Piscataway, NJ), and centrifuged at 1,600
x g for 10 minutes at room temperature. Light-density,
viable bone marrow cells are recovered and washed with 10
10 ml of BSS.FCS. After washing and resuspension in 3 ml of
BSS.FCS, the cells are incubated on ice for 15 minutes
with a mixture of rat monoclonal antibodies (MoAbs),
GK1.5, 53-6.72, 53-7.3.13, RA3-6B2, M1/70.15.11.5, and
RB6-8C5, reactive with specific antigens on mature T, B,
15 and myeloid cells (neutrophils and
monocytes/macrophages). After washing with 10 ml BSS.FCS
and centrifugation through an FCS underlayer, the cells
are resuspended in 1 ml of BSS.FCS, mixed together with 1
ml of prewashed magnetic DYNALBEADS® (Dynalbeads M450;
20 Dynal Inc. Oslo, Norway), and incubated for 20 minutes at
4°C with constant mixing.

        After diluting the mixture by adding 5 ml of fresh
BSS.FCS, the beads together with the cells bound to them,
are removed magnetically. This procedure is repeated
25 twice to both bead and bead-free cell populations. The
bead-free cell fractions (lineage antigen depleted, Lin-)
are pooled, centrifuged, and then stained sequentially at
4°C for 15 minutes each in 0.5 ml of phycoerythrin (PE)-
conjugated goat antirat Ig (mouse absorbed), biotinylated
30 E13-161.7 MoAb (anti-Ly6A/E), and Texas red-avidin. Each
staining step is followed by a washing step with 10 ml of
BSS.FCS.

        The bone marrow cells are preincubated with 50 μg
normal rat Ig for 2 to 3 minutes before the addition of
35 biotinylated E13-161.7 MoAb. After the final wash, the

cells ar resuspended in 3 ml of BSS.FCS containing 10 μg/ml propidium iodide (Calbiochem Corp, La Jolla, CA), and filtered through a 63-μm pore size nylon screen. FACS is performed using an unmodified FACStar-Plus

5 instrument (Becton Dickinson Immunocytometry Systems, San Jose, CA). Cells are positively sorted into Rh123^{lo}, Lin$^-$, Ly6A/E$^+$ (long term repopulating cells of this strain), and Rh$^{med/hi}$, Lin$^-$, Ly6a/E$^+$ (short term repopulating cells of this strain) populations. All

10 cells are kept at 4°C throughout the whole procedure. After completion of cell sorting, the deflected cells are centrifuged at 1,500 rpm for 5 minutes, resuspended, and counted using a hemocytometer.

Induction of Cellular Quiescence

15   Non-limiting examples or procedures for inducing stem cells to become quiescent (enter the G_0 or G_1 phase of the cell cycle) are provided herein. According to the methods of the invention, stem cells are initially induced to enter the cell cycle and expand for the

20 purpose of increasing cell numbers or to provide cellular conditions conducive to retroviral integration. Cellular expansion is induced by the addition of one or more cytokines to the growth medium as described herein.

Prior to transplantation, the expanded stem cells

25 are induced to become quiescent by decreasing the concentration of cytokines, nutrient starvation, addition of inhibitors of cell cycling, or growth on adherent cell cultures. These procedures are described below.

To induce quiescence, cells grown in medium

30 containing a cytokine (such as 50 ng/ml Steel factor alone) or cytokines (a mixture of IL-3 (50 U/ml), IL-6 (50 U/ml), IL-11 (50 ng/ml), and Steel factor (50 U/ml)), are transferred to medium containing 0.5 ng/ml Steel factor as the only cytokine. Cells are monitored for

35 quiescence at days 1, 2, 3, 5, and 7 post-transfer by

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procedures described herein for determining entrance into G_0 or G_1 phase of the cell cycle.

Another procedure for inducing quiescence is to transfer cells growing in medium containing a cytokine (such as 50ng/ml Steel factor alone) or cytokines (a mixture of IL-3 (50 U/ml), Il-6 (50 U/ml), Il-11 (50 ng/ml), and Steel factor (50 U/ml)) to a medium containing the same cytokines but deprived of the nutrient isoleucine. Such nutrient deprivation causes the cells to enter the G_0 or G_1 phase of the cell cycle. Cells are monitored at 1, 2, 5, and 7 days for quiescence as described above.

Yet another procedure for inducing quiescence is to transfer cells grown in the cytokine mixture described above to a medium containing a cell cycle inhibitor such as TGF-β or Macrophage Inhibitor Protein-α (MIP-α). Entrance of the cell population into a quiescent state is monitored as described above.

Induction of a quiescent state is also performed by growing a population of expanded cells on a layer of Dexter culture adherent cells. Such growth condition cause the expanding cells to enter the G_0 of G_1 phase of the cell cycle. Cells are monitored at 1, 2, 5, and 7 days of quiescence and described above.

EXAMPLES

Example 1

Introduction of quiescent stem cells into a mammal is accomplished by injection into a vein of a mammal, e.g., a human, dog, or mouse. Injection volumes in mouse models were the same in each experiment and ranged from 0.5 to 1.0 ml. A portion of the injected stem cells populate the bone marrow, spleen, and thymus. The injected stem cells or transfect d stem cells can be from th host mammal (autologous transplantation) or the c lls

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can be from a diff rent mammal of the same species
(allogenic transplantation).

   An exemplary procedure for assessing engraftment
by the method of the invention was performed as follows.
5 Following the injection of 40x10^6 male BALB/c mouse bone
marrow stem cells for each of five days into female
BALB/c host mice, the percent of male cells or male DNA
in host tissue at various times post transplantation was
determined by standard cytogenetic techniques (Stewart,
10 F.M. et al. (1993) Blood 81:2566-2571). This procedure
allowed identification of male cells as those cells
lacking heterochromatic staining at the centromere (c-
banding).

   Alternatively, Southern analysis using a labelled
15 (e.g., radiolabelled) Y chromosome-specific DNA probe was
used to analyze the percent engraftment of male donor
cells into female host bone marrow as defined above. A
useful Y chromosome-specific probe is the pY2 cDNA probe
(Lamar, E.E. and Palmer, E. (1984) Cell 37:171), or other
20 mouse Y chromosome-specific DNA probe which hybridizes to
repetitive sequences on the murine Y chromosome.

   Southern analysis to detect the degree of
engraftment of male cells into female bone marrow was
performed using standard techniques (see, e.g., Sambrook,
25 et al. (1989) Molecular Cloning: A Laboratory Manual.
Cold Spring Harbor Laboratory Press). Typically, DNA
extracts of murine tissue (such as bone marrow) were
prepared by lysis in 0.15 mol/L NaCl, 0.02 mol/L Tris,
0.02 mol/L EDTA, 1% sodium dodecyl sulfate (SDS),
30 followed by purification using organic extraction with
proteinase K, pancreatic RNAse, and phenol-chloroform
followed by precipitation in ethanol. DNA samples were
analyzed for the presence of Y chromosome-specific
sequences using the pY2 cDNA probe. These samples were
35 digested with the restriction enzymes *DraI* and *BamHI*, and

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the digested fragments were separated by gel
electrophoresis in 0.8% agarose. Equal amounts of
digested DNA, as determined by 260/280 ratios, were
loaded per gel and the comparability of loaded DNA was
5 determined by reprobing membranes with a partial- or
full-length cDNA for interleukin-3 (IL-3) or other marker
CDNA. The DNA fragments were transferred from the gel
onto Zetaprobe nylon membranes (BioRad, Richmond, CA)
according to established Southern blotting techniques.
10 The pY2 probe was labeled with ^{32}P using a random primer
labeling kit (Boehringer Mannheim, Mannheim, Germany);
hybridization with Zetaprobe membranes was performed in
10% dextran sulfate, 1% SDS, 1X Denhardt's, 4X SSCP at
65°C for 18 to 22 hours. Autoradiography was performed
15 using Kodak XRP x-ray film with or without enhancing
screens (Eastman Kodak, Rochester, NY) at -70°C for
varying time intervals. The percentage of male DNA in
female host tissue was calculated based on densitometry
comparisons between post-transplant DNA samples and male
20 murine DNA. Densitometry was performed on an LKB
ultrascan XL enhanced laser densitometer (LKB Pharmacia,
Uppsala, Sweden). Calculations of percent DNA were
adjusted based on amounts of DNA loaded on each gel as
estimated by densitometry of IL-3-probed Southern blots.
25 The detection limit for male DNA in a mixture of male and
female DNA was approximately 3% under these conditions.

Example 2
        Administration of a cell cycle inducing agent
adversely affects the capability of the harvested cells
30 to engraft in a non-myeloablated host prior to
approximately 6 weeks post-transplantation. Bone marrow
harvested from BALB/c mice six days after the mice had
received 150 mg/kg 5-fluorouracil by tail vein injection
was mark dly inferior to normal bone marrow for long term

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engraftment, e.g., greater than approximately 6 weeks
post-transplantation.

In these studies, two tibia/two femur equivalents
(or approximately 40×10^6 bone marrow cells) of male
5   marrow where transplanted by tail vein injection on each
of five consecutive days into non-myeloablated female
hosts. Engraftment was assessed in DNA of host female
splenic and thymic tissues by Southern analysis of pY2
hybridizing sequences at 1, 2, 3, 10, and 20 months
10   following the last transplantation. For host mice
receiving bone marrow from 5-FU-treated male mice, the
mean bone marrow engraftment was 8% at 3 months after
transplantation as compared to 38% for host mice
receiving bone marrow from untreated male mice. This 5-
15   FU treatment defect has also been observed at 3 months
post-transplantation in host female splenic and thymic
tissues.

The engraftment defect seen after 5-FU treatment
is a transient functional defect. Bone marrow cells from
20   5-FU-treated male mice six days after treatment were
found by hydroxyurea suicide analysis to be in active
cell cycle. In contrast, bone marrow cells harvested
from 5-FU-treated male mice 35 days after treatment or
from untreated male mice were not in active cell cycle
25   and exhibited long term engraftment capabilities superior
to those of 5-FU-treated cells (see Table 1).

Table 1

| | % Engraftment | |
	Bone marrow	Spleen
30 Control donor marrow	38.1	33.1
Day 6 post 5-FU	18.1	16.5
Day 35 post 5-FU	35.3	32.9

%Engraftment values were d termined by densitometry of
Southern blots (not corrected for loading on the gel) on
35   4 or 5 mice per group.

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Example 3

This experiment demonstrates that cytokine incubation of murine bone marrow cells impairs the capacity to repopulate in non-myeloablated hosts.

5   Incubation of murine or human hematopoietic cells with various cytokine combinations has been used to expand stem cell populations and induce stem cell replication resulting in enhanced gene-carrying retroviral integration. This study examined the effect of a 48 hour

10   in vitro incubation in cell culture medium (DMEM-Low glucose, 15% fetal calf serum) with a cytokine combination of murine IL-3-CHO (50 U/ml), murine IL-6 (50 U/ml), murine IL-11 (50 ng/ml), and murine SCF (50 U/ml) on engraftment of a first group of male donor bone marrow

15   cells into non-myeloablated female BALB/c host mice. A second group of donor cells was incubated with medium alone, and a third group of donor cells was not incubated (Fig. 2).

In each group three injections of 40×10^6 cells per

20   injection were made over three days. Engraftment was evaluated by Southern analysis using a Y chromosome-specific probe (pY2). Mean engraftment of male donor bone marrow cells into female BALB/c host mice was determined (y axis). For Group 1 host mice, donor cells

25   were incubated for 48 hours in culture medium plus IL-3, IL-6, IL-11, and SCF. Three injections each of 40×10^6 cells were given to host mice. For Group 2 host mice, donor cells were incubated for 48 hours in culture medium alone. Three injections each of 40×10^6 cells were given to host mice. For Group 3 host mice, three injections

30   each of 0.5 ml PBS (phosphate buffered saline lacking cells) were given to host mice. Donor cells administered to Group 4 mice were not incubated prior to giving three injections each of 40×10^6 cells to host mice.

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Neither the cytokine-incubation group, nor the medium-only incubation group demonstrated evidence of male DNA sequence, indicating that no engraftment occurred (Fig. 2). The control group which received

5   cells immediately after donor collection, showed a 16.6% engraftment level (range 11.6-24.8%, n=4) in host bone marrow. This result is consistent with the previous observation that pre-treatment with a cell cycle inducing agent, 5-FU, causes decreased engraftment, and indicates

10 that exposure to cytokines and/or inducement of active cell cycling impairs engraftment and repopulating capacity of hematopoietic stem/progenitor cells in non-myeloablated hosts.

A similar cytokine-induced impairment of

15 repopulating capacity was observed for cells from C57BL/6 (Ly5.2) mice and B6.SJL-Ly5a(Ly5.1) mice. Figs. 3A and 3B show the progenitor cell content present in 10×10^4 noncultured (0 hours) cells, and that of culture flasks initiated with 10×10^6 cells after 48 hours of culture.

20 CPP-CFC are represented by dark shaded bars (■) at the bottom of the columns, and LPP-CFC are represented as lighter bars (□) at the top of the columns. Cultured marrow cells from C57BL/6 (Ly5.2) were expanded by 49% for HPP-CFC and 14% for LPP-CFC (Fig. 3A). Cells from

25 B6.SJL-Ly5a (Ly5.1) were expanded by 206% for HPP-CFC and 166% for LPP-CFC (Fig. 3B). This expansion of noncultured progenitors by cytokine-treatment was significant for HPP-CFC and LPP-CFC in Ly5.1 marrow (P = .01, Wilcoxon's rank sum test), and had a significance

30 level of P = .1 in Ly5.2 marrow.

Example 4

Cytokines are used for the *in vitro* expansion of hematopoietic progenitor/stem c lls resulting in an increased percentag of stem cells in active cell

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cycling. An evaluation of the level of engraftment as a
function of the cell cycle in which the population of
donor cells reside was performed.

    Male BALB/c donor bone marrow cells were exposed
5 *in vitro* to a combination of cytokines for enhancement of
retroviral integration *in vitro* in primitive
progenitor/stem cells. Male BALB/c mouse bone marrow was
incubated in culture media alone or in media plus IL-3
(50 U/ml), IL-6 (50 U/ml), IL-11 (50 ng/ml), and steel
10 factor (c-Kit ligand, 50 U/ml; Genetics Institute,
Cambridge, MA) for 6, 24, or 48 hours (x axes of Figs. 1A
and 1B). High proliferative potential colony-forming
cells (HPP-CFC) and total progenitors (>50 cell colonies)
were determined per culture flask (y axes of Figs. 1A and
15 1B). Cell cycle status was assessed by determining the
percentage of cells traversing S phase (synthesizing DNA)
by treating the cells *in vitro* with tritiated thymidine
(^{3}HtdR) and determining the percentage of cells killed
(Figs. 1A and 1B).

20   Donor cells were also evaluated for the effects of
in vitro liquid culture on HPP-CFC (multi-factor
responsive, Fig. 1A) and total progenitors (Fig. 1B). At
initiation of the cultures, there was less than 3.5%
killing in both the cytokine-exposed and media-only cell
25 cultures indicating that the donor cells were in the
quiescent (G_1 or G_0) phase (Figs. 1A and 1B). There was a
progressive increase in killing at 6, 24, and 48 hours
(Figs. 1A and 1B) in the cytokine-treated cell cultures.
Donor bone marrow cells incubated *in vitro* with cytokines
30 show a maintenance of multi-factor responsive HPP-CFC
(111% of input, Fig. 1B) and an expansion of total
progenitors (199% of input, Fig. 1A) with a concomitant
progression from quiescence to active cell cycling.
Initial ^{3}HtdR killing was 3.5% and 1.0% of HPP-CFC (Fig.
35 1B) and total progenitor (Fig. 1A), respectively. Aft r

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48 hours the proportions of cells killed by exposure to
^{3}HtdR were 60% (Fig. 1B) and 58% (Fig. 1A), respectively.

Cell-cycle analysis (thymidine suicide) of
noncultured marrow and progenitor cells from C57BL/6 and
5 B6.SJL/Ly5a mice, in which the cells were incubated for
48 hours in culture medium with the cytokines mSCF, IL-3,
IL-6, and IL-11 is illustrated in Figs. 4A and 4B. Bar
graphs represent HPP-CFCs and LPP-CFCs present in 10 x
10^4 noncultured (0 hours) marrow cells and in 10 x 10^4
10 cultured marrow cells (48 hours) either short-term
exposed (dark bars) or not exposed (light bars) to high
activity tritiated thymidine (^{3}HTdR). HPP-CFC data are
plotted in front, LPP-CFC data at the back.

The thymidine suicide assay of C57BL/6 (Ly5.2)
15 progenitors showed no killing of LPP-CFCs and of HPP-CFCs
at zero time, indicating that these progenitor cell
populations were dormant (Fig. 4A). After 48 hours of
culture with cytokines 86% of LPP-CFC and 95% of HPP-CFC
were killed and thus progressing through S phase. The
20 thymidine suicide assay of B6.SJL-Ly5a (Ly5.1)
progenitors showed little kill of noncultured LPP-CFCs
(32%) and HPP-CFCs (20%) (Fig. 4B). After 48 hours of
culture with cytokines, 86% of LPP-CFC and 95% of HPP-CFC
were killed and thus in cell-cycle progression. They had
25 a mean of 0% in noncultured Ly5.2 (C57BL/6) marrow (Fig.
4A) and 20% in noncultured Ly5.1 (86-SJL-Ly5a) marrow
(Fig. 4B). After culture with cytokines a mean of 95%
and 98% of HPP-CFC were killed by exposure to high-
activity tritiated thymidine in either strain, indicating
30 that they had been induced to enter active phases of the
cell cycle. That meant LPP-CFC killed was 0% in
noncultured Ly5.2 marrow (Fig. 4A) and 32% in noncultured
Ly5.1 marrow (Fig. 4B). Culture with cytokines induced
killing at a mean of 86% of LPP-CFC in both strains. A
35 standardized-normal test comparing the mean differences

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of noncultured and cultur d progenitors was highly
significant (^{P}<.0001) in both strains.

Induction of stem cell expansion using steel
factor alone at 50 ng/ml for 24-48 hours provides the
5   same degree of cellular expansion as the combination of
·IL-3, IL-6, IL-11 and steel factor (data not shown).

Example 5

To determine the efficiency of engraftment of
treated cells into a non-myeloablated host by scheduled
10 administration over time, the following experiment was
performed. Four groups of male mouse donor bone marrow
cells were evaluated for engraftment into normal non-
myeloablated BALB/c female mice. Staggered liquid marrow
cultures were established and female BALB/c non-
15 myeloablated hosts were transplanted with 40x10^6 non-
cultured male BALB/c bone marrow cells for three
consecutive days (total of 120x10^6 cells) or the
equivalent of 40x10^6 cultured cells exposed or not
exposed to the cytokine regimen described above. At 35
20 days following the last transplantation, engraftment was
evaluated by Southern blot analysis using the pY2 probe
or by FISH analysis (Fluorescence In Situ Hybridization
analysis, a standard technique well known to those of
ordinary skill in the relevant arts). Non-cultured donor
25 bone marrow cells showed a mean engraftment of
approximately 15% while no engraftment was observed with
cells cultured in media alone or media with cytokines
(IL-3, IL-11, IL-6, and steel factor).

Example 6

30   Exposure of donor bone marrow cells to cytokines
is useful to enhance retroviral integration for the
formation of transfected cells. The efficiency of

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engraftment of transfected cells was examined to evaluate
the usefulness of such cells in a gene therapy regimen.

Male BALB/c bone marrow cells were transfected
with a retroviral vector carrying the MDR gene to produce
5 transfected cells. The transfection protocol involved
inducing the cells into cell cycle by cytokine exposure
and provided over 90% MDR transfection into the primitive
stem cells (HPP-CFC). The transfected cells were
transplanted into female BALB/c mice on 5 consecutive
10 days (one transplantation per day for a total of 45 x 10^6
cells transplanted over 5 days). Following the last
transplantation, the mice were treated three times with
Taxol (10 mg/kg IP) over 7 months to expand the MDR-
carrying stem cells in vivo. At 14 months following
15 transplantation, the recipient mice were sacrificed for
analysis of engraftment and the presence of the MDR gene.

There was a 8.9% to 13.4% engraftment of male
transfected cells into female hosts as determined by
Southern analysis using the pY2 probe. However, there
20 was only minimal expression in 3 of 4 transplanted
animals of the MDR gene as determined by RT-PCR (reverse
transcriptase-polymerase chain reaction). Thus, cycling
murine stem cells successfully integrated the MDR gene
following retroviral infection in vitro, but very few of
25 the cells which engrafted were carrying the gene. This
suggests that dormant G_0 cells without the retroviral
gene construct were able to engraft, and that a critical
feature of successful engraftment is the use of dormant
cells. Effective gene transfer requires that the stem
30 cells be returned to a quiescent state prior to
transplantation.

Example 7

This example describes the ability to enhance
engraftment by administering non-cycling cells. Th

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competitive assay used cells from Ly5.2/Ly5.1 mice which
allow engraftment to be monitored by immunoassay for the
presence of cells having the Ly5.2 cell surface marker.

     Bone marrow cells were cultured with cytokines for
5 48 hours to induce cellular expansion. Bone marrow cells
were obtained by flushing tibias and femurs of 6- to 8-
week-old Ly5.2 (C57BL/6) and Ly5.1 (B6.SJL-Ly5a) mice.
Ten million bone marrow cells were cultured in
nonadherent tissue culture dishes at a density of 10^6
10 nucleated cells/ml in DMEM (low glucose), supplemented
with 15% heat-inactivated fetal calf serum (FCS) with
cytokines (recombinant mouse SCF (rmSCF) at 50 ng/ml,
rmIL-3 at 60 U/ml, rmIL-6 at 50 U/ml, and recombinant
human IL-11 (rhuIL-11) at 50 ng/ml) at 37°C in a fully
15 humidified atmosphere of 5% CO_2 in air. After 48 hours
all cultured cells were procured by vigorous pipetting
with ice-cold phosphate-buffered saline (PBS) and cell
scraping (as determined by microscopy).

     The cell cycle status was analyzed by comparing
20 colony growth of marrow exposed to high activity
tritiated thymidine (^{3}H-TdR) to colony growth of marrow
that was not challenged with radioactivity. The
percentage of HPP-CFCs and LPP-CFCs killed by exposure to
^{3}H-TdR indicate the fraction of HPC that underwent active
25 DNA synthesis in cell cycle progression. No cell cycle
activity could be detected in noncultured Ly5.2 (C57BL/6)
marrow. The amount of HPP-CFC and LPP-CFC killed in
noncultured fresh marrow by thymidine suicide was a mean
of 0% indicating that most HPC were in dormancy. After
30 culture with cytokines a mean of 95% of HPP-CFCs and a
mean of 86% of LPP-CFCs were killed by exposure to high-
activity tritiated thymidine in these Ly5.2 mice (Fig.
4A). This indicates that most of the cytokine-treated
cells had been induced to enter active phases of the cell
35 cycle.

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This change of HPC cycle status induced by culture of marrow cells with cytokines was also observed in marrow from Ly5.1 (B6.SJL-Ly5a) mice (Fig. 4B). In this strain 20% of HPP-CFCs and 32% of LPP-CFCs of noncultured

5 marrow were killed by exposure to ^{3}H-TdR. The amount of progenitor cells entering active phases of the cell cycle increased dramatically as shown by HPP-CFC fractions of 98% and LPP-CFC fractions of 86% killed by exposure to high activity tritiated thymidine in this strain. A

10 standardized-normal test comparing the mean differences of noncultured and cultured progenitors was highly significant (P < 0.0001) in both strains.

   Expansion of HPC was determined by comparing the contents of HPP-CFCs and LPP-CFCs of both freshly

15 obtained uncultured marrow and cytokine-treated cells in a soft-agar colony assay. Cultured cells were procured by vigorous pipetting with ice-cold PBS and cell scraping until virtually no residual cells remained in the culture flasks as determined by microscopy. Cultured marrow

20 cells from Ly5.2 (C57BL/6) and Ly5.1 (B6.SJL) mice were expanded by 49% and 206% for HPP-CFC (dark bars, Figs. 3A and 3B, respectively), and 14% and 166% for LPP-CFC (light bars, Figs. 3A and 3B, respectively) in these murine strains. This expansion of cultured progenitors

25 by cytokine-treatment was significant for HPP-CFC and LPP-CFC in Ly5.1 marrow (P<.01. Wilcoxon's rank sum test). The significance level for Ly5.2 marrow was P = 0.1.

   A competitive Ly5.2/Ly5.1 transplantation assay

30 was performed as follows. Recipient mice were exposed to 12.0 Gy (7.0 Gy and 5.0 Gy given 3 hours apart) in a gamma cell 40 cesium source and 1 hour later injected intravenously (IV) with (1) a mixture of 50% cytokine-cultured Ly5.1 c lls (equivalent to 20 x 10^6 starting

35 cells procured after 48 hours of incubation with

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cytokin s) and 50% fresh noncultured Ly5.2 cells; (2) a
mixture of 50% noncultured Ly5.1 cells (equivalent to 20
x 10^6 starting cells harvested after 48 hours of
incubation with cytokines) and 50% cytokine-treated Ly5.2

5   cells; and (3) a mixture of 50% cytokine-cultured cells
(equivalent to 20 x 10^6 starting cells harvested after 48
hours of incubation with cytokines) of both cell types.
Volumes for injection ranged from 0.5 to 1.0 ml. The
percentages of marrow cells bearing the Ly5.2 and Ly5.1

10 phenotypes were determined 12 and 22 weeks post-
transplant by fluorescence-activated cell sorter (FACS)
analysis. One million suspended cells were incubated for
10 minutes with mouse 1gG (Sigma Chemicals, St. Louis,
MO, USA) for 30 minutes at 4°C. The Ly5-specific

15 monoclonal antibodies for Ly5.2 (fluorescein
isothiocyanate [FTTC]-conjugated monoclonal antibody
1042.1) and Ly5.1 (biotin-conjugated A201.7) (Jackson
Immunolaboratories) were added for 30 minutes, and then
washed twice. Cell-bound biotinylated monoclonal

20 antibody A201.7 was detected with streptavidin-Red 670
(R&D, Minneapolis, MN, USA), which was incubated for 30
minutes at 4°C. Cells were washed twice and subsequently
analyzed with an FACS (FACS-STAR:Becton Dickinson
Immunosystems, San Jose, CA).

25   Bone marrow engraftment was assessed after
competitive transplantation of noncultured with cultured
marrow cells by FACS analysis for donor-derived cells
stained with monoclonal antibodies specific for the two
alleles of Ly-5 (Figs. 5A to 5C). Columns represent the

30 marrow fraction of individual mice positive for anti-
Ly5.2 antibody staining. Analysis of anti-Ly5.1 antibody
straining gave reciprocal results (data not shown). Fig.
5A represents analysis at 12 weeks post-transplantation
in Ly5.1 (B6.SJL-Ly5a) recipients; noncultured Ly5.2

35 (C57BL/6) marrow competed with Ly5.1 marrow led to mean

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(±SE) engraftment levels of 70% ± 1% (n=10) for the Ly5.2
cells; cytokine-cultured Ly5.2 marrow cells competing
with Ly5.1 cells led to 5% ± 1% (n=10) engraftment.
Differences in engraftment between noncultured and
5 cytokine-treated populations were significant (P<.01,
Wilcoxon's rank sum test). Fig. 5B represents analysis
at 12 weeks post-transplantation in recipient mice of
Ly5.2 background. Noncultured Ly5.2 cells competing with
Ly5.1 cells led to 63% ± 5% (n=5) engraftment for the
10 Ly5.2 cells; cultured Ly5.2 cells competing Ly5.1 cells
contributed to 7% ± 2% (n=5) engraftment. Differences in
engraftment were significant at a level of P = 0.1.
Analysis at 22 weeks post-transplantation in recipient
mice of Ly5.1 background is shown in Fig. 5C.
15 Noncultured Ly5.2 marrow cells contributed a mean of 93%
± 2% (n=10) to engraftment whereas cytokine-treated Ly5.2
cells gave a mean engraftment of 4% ± 2% (n=10) when
compared with Ly5.1 marrow cells. Differences in
engraftment between noncultured and cytokine-treated
20 populations were significant at 22 weeks post-
transplantation (P < 0.01).

Example 8
        A cytokine-induced engraftment defect was observed
when cytokine treated and untreated cells from male and
25 female mice were administered to a myeloablated host.
Engraftment was assessed by monitoring the presence of
male stem cells administered to a female myeloablated
host using the Y chromosome as a marker.
        Cells obtained from BALB/c mice were cultured as
30 described above for the Ly5.2/Ly5.1 competitive assay.
Female recipient mice were given 10.5 Gy irradiation in
doses of 5.5 Gy and 5.0 Gy administered 3 hours apart and
1 hour later injected intravenously with eith r a mixture
of 50% cytokine-cultured female (corresponding to th

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equivalent of 20 x 10^4 starting cells harvested after 48
hours of culture with cytokines) and 50% (20 x 10^4) fresh
noncultured female cells. Volumes for injection were the
same in each experiment and ranged from 0.5 to 10 ml.
5 The percentage of male marrow in host marrow cells was
then determined 10 and 14 weeks post-transplantation
using Southern blot analysis with the Y-chromosome-
specific pY2 probe (Lamar, E.E. and Palmer, E., Cell
37:171, 1984) and performed as described (see Example 1).
10   Briefly, approximately 5 μg of genomic DNA per
sample was digested with the restriction enzyme Dra-1
(Boehringer Mannheim, Indianapolis, IN) and separated by
gel electrophoresis in 0.8% agarose (GIBCO-BRL). DNA
fragments were transferred onto Zetaprobe nylon membranes
15 (BioRad, Richmond, CA) by capillary transfer overnight in
0.4 N sodium hydroxide. The blots were probed for the
presence of Y-specific sequences using pY2-cDNA.
Autoradiography was performed on Kodak XRP films (Eastman
Kodak, Rochester, NY). The percentage of radioactivity
20 of male and female DNA was quantified by exposing filters
to photostimulatable phospor imaging plates (Molecular
Dynamics, Sunnyvale, CA) and scanning the plates with a
400 Å PhosphorImager (Molecular Dynamics) (Johnston, R.F.
et al., Electrophoresis 11:355, 1990) and quantitation
25 was based on comparisons between post-transplant DNA
samples and male murine DNA. Calculations of percent DNA
were adjusted based on amounts of DNA loaded on each gel
as determined by separate probing with a cDNA which
hybridizes specifically to IL-3.
30   A competitive transplant assay was performed using
the equivalent of 20 X 10^6 starting male or female cells
obtained after a culture with the cytokines mSCF, IL-3,
IL-6, and IL-11. Cells subjected to cytokine treatment
for 48 hours were progressing through the cell cycle (see
35 Example 4, supra). The cells were mixed and inject d

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together with fresh, noncultured cells of the opposite
gender into irradiated (10.5 Gy) recipient mice.
Southern blot engraftment analysis using the Y-
chromosome-specific pY2 cDNA probe showed compromised
5 engraftment of cultured cytokine-exposed marrow cells
competing with noncultured marrow cells in bone marrow,
spleen, and thymus of lethally irradiated female hosts at
10 and 14 weeks post-transplantation. Noncultured male
BALB/c marrow cells, competing with cytokine-cultured
10 female BALB/c marrow, lead to mean engraftment (±SE)
levels of 70% ± 2% (n = 5) in female BALB/c recipient
marrow at 10 weeks post-transplantation. Mean
engraftment levels of cultured male marrow cells
competing with noncultured female cells were 13% ± 9% (n
15 = 5) in the marrow of female recipients at 10 weeks.
This difference in engraftment of noncultured and
cytokine-treated populations is statistically significant
at a level of P = 0.5 (Wilcoxon's rank sum test). The
engraftment defect was greater 14 weeks after
20 transplantation. Fresh noncultured male BALB/c marrow
cells competing with cytokine-treated female cells
contributed to 95% ± 9% (n = 10) marrow engraftment while
cytokine-treated male cells competing with noncultured
female cells gave a mean level of only 2% ± 1% (n = 10).
25 Differences in engraftment between non-cultured and
cytokine-treated populations were highly significant (P <
0.01). In these BALB/c transplants the engraftment
defect was also apparent when spleen and thymus were
analyzed, indicating that this is a general engraftment
30 defect.
        Studies using both engraftment models showed an
increased engraftment defect at longer time intervals
post-transplantation, suggesting that the most striking
effect may be on long-term repopulating stem c lls.

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    In the transplant models involving lethal whole-
body irradiation, the possibility of a contribution from
host marrow repopulation was excluded. Irradiated femal
mice were injected with male cells and repopulation was
5 by male cells only. Similar controls werE performed and
results obtained in the Ly5.2/Ly5.1 model.

    Statistical analysis was performed using levels of
significance of expansion of non-cultured progenitors by
cytokine-treatment analyzed by Wilcoxon's rank sum test
10 (Freund, J.E. and Walpole, R.E., Mathematical Statistics
(ed. 3). Englewood Cliffs, NY, Prentice-Hall, 1980, p.
478). Mean differences of percentages of noncultured and
cultured progenitors killed by in vitro [3]HTdR exposure
were compared by standardized-normal tests (Freund, J.E.
15 and Walpole, R.E., Mathematical Statistics (ed. 3).
Englewood Cliffs, NY, Prentice-Hall, 1980, p. 478).
Differences in engraftment of noncultured and cytokine-
treated populations were compared by Wilcoxon's rank sum
test.

20 Example 9

    Preparation of quiescent stem cells for
transplantation can be accomplished by a number of
different techniques. In a first method, noncycling stem
cells are isolated from a mammal which has not been
25 treated with a cell cycle inducing agent for a period of
time sufficient to allow at least 50%, preferably at
least 70%, most preferably at least 80% of the stem cells
to return to the quiescent G_1 or G_0 phase of the cell
cycle. Such quiescent bone marrow stem cells are used
30 directly for transplantation into a non-myeloablated
mammal.

    Actively cycling stem cells can be isolated from a
mammal treated with a cell cycling inducing agent or the
harvested stem cells can be treated in vitro with a cell

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proliferation agent, e.g., a cytokine, to increase the
number of stem cells or to enhance *in vitro* retroviral
infection for use in *ex vivo* gene therapy. Subsequently,
actively cycling stem cells are induced to become
5   quiescent by removing nutrients from the culture medium,
e.g., serum starvation, causing at least 50%, preferably
at least 70%, more preferably at least 80% of the cells
to enter the G_0 cell cycle phase. The quiescent stem
cell population is then transplanted into a host mammal.

10 Example 10
        The efficiency of engraftment in the first weeks
post-transplantation was examined using the BALB/c mouse
model described herein. Bone marrow from male BALB/c
mice was incubated for 48 hours in the presence of IL-3,
15 IL-6, IL-11, and Steel factor, as described above. These
cells were mixed in approximately equal proportions with
untreated female BALB/c mouse bone marrow. The mixture
was administered to female BALB/c mice which had been
lethally irradiated (1100 rads). As indicated herein,
20 such conditions led to very low efficiency of engraftment
of the male cytokine-treated cells when observed after 10
or more weeks post-transplantation. However, when
engraftment was observed at one week post-
transplantation, engraftment of the cytokine-treated
25 cells was superior to untreated cells.
        Treatment of the early repopulating stem cells
with a cytokine results in cell expansion. Cytokine-
treated stem cells engraft with high efficiency during
the first approximately 6 weeks, and as early as one
30 week, after engraftment. Thus, stimulating early
repopulating stem cells to enter the cell cycle enhances
their ability to engraft, making such cells useful for
transplantation or *ex vivo* gene therapy requiring early

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(and short-term) engraftm nt of the cells, e.g., for gene
therapy to treat transient conditions such as tumors.

Cytokine-treated stem cells exhibit very low long
term engraftment. In the preceding examples, untreated
5   cells were found in the engrafted population, while no
detectable cytokine-treated cell progeny were observed at
22 weeks post-transplantation. Cells known to be
quiescent were shown herein to engraft at a higher
efficiency relative to cytokine-treated cells when long-
10 term engraftment is monitored, where long term is a time
greater than approximately 6 weeks post-transplantation.

A method of maximizing efficiency of short and
long term engraftment is an embodiment of the present
invention. Bone marrow replacement or the effects of
15 gene therapy are rapidly achieved by maximizing short
term engraftment. Maximizing long term engraftment is
useful in patient treatment situations which require
extended effects such as in long term gene therapy.

Short term engraftment is achieved in a host
20 mammal by treating stem cells appropriate for engraftment
with a cytokine or combination of cytokines to induce
proliferation as described herein. If gene therapy is a
desired treatment, the cells are transfected with an
exogenous gene of interest while actively progressing
25 through the cell cycle. While the cytokine-treated cells
continue to progress through the cell cycle, a fraction
of the cells are administered to a myeloablated or non-
myeloablated host mammal. Cells treated in this manner
engraft with high efficiency and remain in the bone
30 marrow population during the approximately first 6 weeks
post-transplantation.

According to the method of the invention, the
remaining fraction of the cells are induced to become
quiescent prior to transplantation. As described herein,
35 cells which are not actively cycling ngraft at a high

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efficiency at times greater than approximately 6 weeks
post-transplantation.

As a result of such administration of cycling and
non-cycling stem cells, both early and long term effects
5 bone marrow transplantation or gene therapy are achieved,
enhancing the ability to effectively treat the patient.
Long term gene therapy is more suited for genetic defects
or enzymatic deficiencies.

Alternatively, populations of stems cells enriched
10 in short or long term repopulating stem cells may be
obtained and these populations administered to the host
mammal to maximize the efficiency of short or long term
engraftment. Such a method would include first obtaining
a population of pluripotent hematopoietic stem cells and
15 inducing the population of cells to expand. The early
repopulating stem cells are separated from the late
repopulating stem cells by a stem cell sorting technique
such as the technique described above under General
Procedures. The population enriched with actively cell
20 cycling early stem cells is introduced into the host
mammal if short term engraftment is desired. The
population enriched with late repopulating stem cells is
induced to become quiescent prior to introduction into
the host mammal if long term engraftment is desired.

25   The above method is used for *ex vivo* gene therapy
by including the step of transfecting the expanded cells
with exogenous DNA by retroviral integration, for
example, prior to the stem cell separation step.

Use

30   Diseases that are treated by transplantation of
bone marrow stem cells and for which treatment can be
enhanced by the methods of the invention include, but are
not limited to, diseases such as lymphoma, multipl
myeloma, breast cancer, testicular cancer, leukemias,

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cong nial hemolytic anemias (sickle cell anemia and
thalassanemia) and some immunodeficiency diseases (e.g.,
acute leukemia, Hodgkin's and non-Hodgkin's lymphoma, and
neuroblastoma).

5   Several human diseases can be treated by *ex vivo*
gene therapy involving transplantation of transfected
bone marrow stem cells, and such treatment can be
enhanced by the methods of the invention. Correction of
human inherited disorders in bone marrow stem cells in
10 tissue culture has been shown for Gaucher disease in
which the mutant glucocerebrosidase-encoding gene is
replaced by the wild type gene. Dihydrofolate reductase
deficiency in which a mutated form of the dihydrofolate
reductase-encoding gene (DHFR) is replaced has been
15 performed in mice (Watson, J.D. et al., *Recombinant DNA*,
2nd ed., pp. 571-576).

Ex vivo gene therapy in large animals such as
humans presents several difficulties that can be
alleviated by enhancement of stem cell engraftment
20 following transplantation of quiescent cells. In one set
of studies using macaque monkeys, bone marrow cells from
the monkeys were infected with a vector carrying a human
cDNA for adenosine deaminase. The donor monkeys received
total body irradiation and then an infusion of infected
25 cells. Only low levels of expression of human adenosine
deaminase-encoding cDNA could be detected in these
mammals. Similar results were obtained in experiments
with bone marrow cells that carried the human β-globin
gene (Watson, J.D. et al., *Recombinant DNA*, 2nd ed., pp.
30 571-576). Such reduced expression can be enhanced by the
method of the invention in which quiescent transfected
stem cells are transplanted into the non-myeloablated
recipient mammal. Finally, transfer of a normal human
adenosine deaminase gene (ADA) into a human patient for
35 the treatment of severe combined immune deficiency caused

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by a mutation in ADA has been achiev d with ncouraging
results using bone marrow-stem cell gene therapy in
humans (Watson et al. (1992), supra, p. 578). However,
the treatment required that the patients receive gene-
corrected cells every 1 to 2 months for up to one year
before showing signs of clinical improvement. The
instant invention is useful in enhancing the engraftment
at each treatment and thereby more quickly and assuredly
improve the health of the patient.

What is claimed is:

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Claims

1. A method of preparing a stem cell composition
for transplantation into a host mammal, said method
comprising the steps of:
5   a) providing a population of pluripotent
hematopoietic stem cells;
           b) expanding said population of stem cells to
yield a population of expanded stem cells; and
           c) treating said population of expanded stem cells
10 to induce a majority of the cells in said population to
become quiescent, and using said quiescent stem cells as
the cell composition.

2. A method of preparing a stem cell composition
for use in *ex vivo* gene therapy in a host mammal, said
15 method comprising the steps of:
           a) providing a population of pluripotent
hematopoietic stem cells;
           b) expanding said population of stem cells to
yield a population of expanded stem cells;
20   c) transfecting said expanded stem cells with
exogenous DNA to generate a population of transfected
stem cells; and
           d) treating said population of transfected stem
cells to induce a majority of the cells in said
25 population to become quiescent, and using said quiescent
transfected stem cells as the cell composition.

3. A method of preparing a stem cell composition
for use in long term *ex vivo* gene therapy in a host
mammal, said method comprising the steps of:
30   a) providing a population of pluripotent
hematopoietic stem cells;

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b) expanding said population of st m cells to
yield a population of expanded stem cells;

c) transfecting said expanded stem cells with
exogenous DNA to generate a population of transfected
5 stem cells, said population comprising early repopulating
stem cells and late repopulating stem cells;

d) separating said early repopulating stem cells
from said late repopulating stem cells; and

e) treating said transfected late repopulating
10 stem cells to induce a majority of the cells in said
population to become quiescent, and using said quiescent
transfected late repopulating stem cells as the cell
composition.

4.   The method of any one of claim 1, 2, or 3,
15 wherein said stem cell composition is administered to the
host mammal in a series of administrations, wherein

i) a first portion of said stem cell composition
is administered to the host at a first point in time; and

ii) thereafter, additional portions of said stem
20 cell composition are administered in at least five and
not greater than fifteen additional administrations, each
spaced from the other by at least 12 hours.

5.   A method of preparing a stem cell composition
comprising first and second dosage units for
25 transplantation into a host mammal to provide short term
and long term engraftment of said stem cells, said method
comprising the steps of:

a) providing a population of pluripotent
hematopoietic stem cells;

30   b) expanding said population of stem cells to
yield a population of expanded stem cells;

c) dividing said population of expanded stem c lls
into a first fraction and a second fraction, and using

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said first fraction as the first dosage unit of th stem
cell composition; and

     d) treating said second fraction of said
population of expanded stem cells to induce a majority of
5 the cells in said fraction to become quiescent stem
cells, and using said quiescent stem cells as the second
dosage unit of the stem cell composition.

     6.   A method of preparing a stem cell composition
comprising first and second dosage units for use in *ex
10 vivo* gene therapy in a host mammal, said method
comprising the steps of:

     a) providing a population of pluripotent
hematopoietic stem cells;

     b) expanding said population of stem cells to
15 yield a population of expanded stem cells;

     c) transfecting said expanded stem cells with
exogenous DNA to generate a population of transfected
stem cells;

     d) dividing said population of transfected stem
20 cells into a first fraction and a second fraction, and
using said first fraction as the first dosage unit of the
stem cell composition; and

     e) treating said second fraction of transfected
stem cells to induce a majority of the cells in said
25 second fraction to become quiescent transfected stem
cells, and using said second fraction as the second
dosage unit of the cell composition.

     7.   The method of any one of claims 1 through 6,
wherein the host mammal is a non-myeloablated mammal.

30   8.   The method of any one of claims 1 through 6,
wherein the host mammal is a myeloablated mammal.

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9.   The method of any one of claims 1 through 8,
wherein said stem cells are selected from the group
consisting of allogenic bone marrow cells, autologous
bone marrow cells, peripheral-blood progenitor cells, and
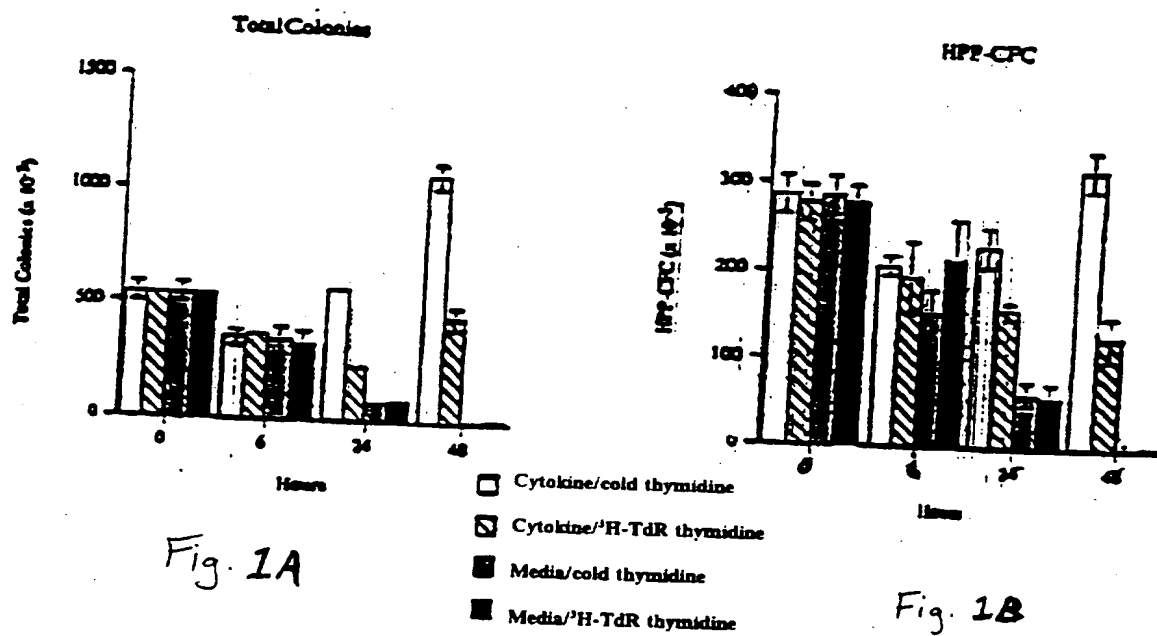5 peripheral-blood bone marrow cells.

10.   The method of any one of claims 1 through 8,
wherein said stem cells are expanded by contacting said
cells with a cytokine under conditions for cell
proliferation.

10   11.   The method of claim 10, wherein said cytokine
is selected from the group consisting of Steel factor,
IL-3, IL-11, and IL-6, or any combination thereof.

12.   The method of claim 10, wherein said cytokine
is Steel factor.

15   13.   A stem cell composition for use in
transplantation into a host mammal, said composition
comprising a population of pluripotent hematopoietic stem
cells, wherein said population is first induced to
expand, and is thereafter treated to induce a majority of
20 the cells in said population to become quiescent, prior
to transplantation into the host mammal.

14.   A stem cell composition for use in *ex vivo*
gene therapy in a host mammal, said composition
comprising a population of pluripotent hematopoietic stem
25 cells transfected with exogenous DNA, wherein said
population is first induced to expand, and is thereafter
treated to induce a majority of the cells in said
population to become quiescent, prior to transplantation
into the host mammal.

15. A stem cell composition for use in long term *ex vivo* gene therapy, said composition consisting essentially of a population of late repopulating pluripotent hematopoietic stem cells transfected with 5 exogenous DNA, wherein said population is first induced to expand, and is thereafter treated to induce a majority of the cells in said population to become quiescent, prior to transplantation into the host mammal.

16. A stem cell composition for use in 10 transplantation or *ex vivo* gene therapy in a host mammal, said composition comprising two dosage units, wherein said first dosage unit consists essentially of a population of expanded stem cells, and said second dosage unit consists essentially of a population of stem cells 15 that is first induced to expand, and thereafter treated to induce a majority of the cells in the population to become quiescent, prior to administration to the host mammal.

17. The composition of any one of claims 13, 14, 20 15, or 16, wherein said stem cells are selected from the group consisting of allogenic bone marrow cells, autologous bone marrow cells, peripheral-blood progenitor cells, and peripheral-blood bone marrow cells.

18. The composition of any one of claims 13, 14, 25 15, 16, or 17 wherein said stem cells are expanded by contacting said cells with a cytokine under conditions for cell proliferation.

19. The composition of claim 18, wherein said cytokine is selected from the group consisting of Steel 30 factor, IL-3, IL-11, and IL-6, or any combination ther of.
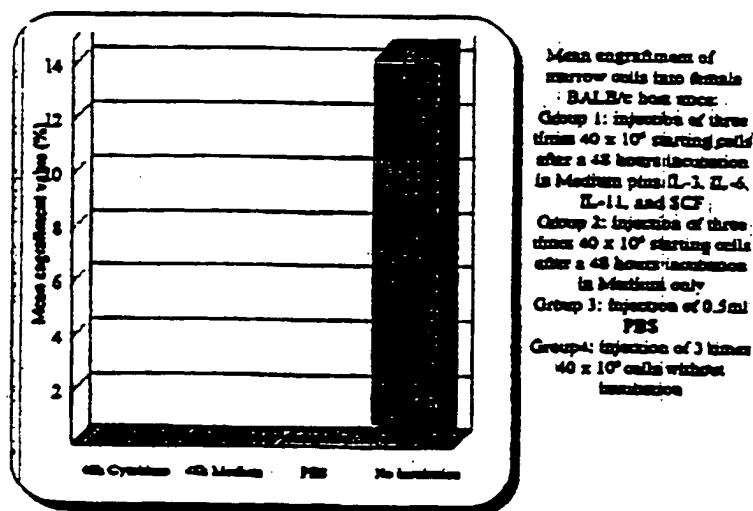
20.   The composition of claim 18, wh rein said
cytokine is Steel factor.

Fig. 1A

Fig. 1B

☐ Cytokine/cold thymidine

▨ Cytokine/³H-TdR thymidine

▦ Media/cold thymidine

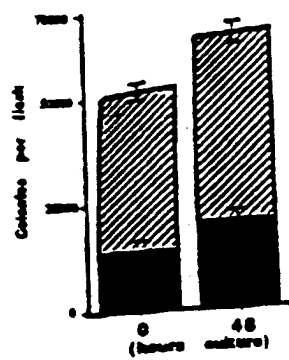■ Media/³H-TdR thymidine

Bone marrow engraftment into non-myeloablated hosts.

Bone marrow

Influence of a preincubation of donor marrow cells in growth medium
containing the cytokines IL-3, IL-6, IL-11, and SCF

Southern blot hybridization with pY2 DNA probe. Radio-analytic-imaging.
Values subsequently corrected for loading variation

Fig. 2

C57BL/6 (Ly5.2)

Fig. 3A

B6.SJL (Ly5.1)

Fig. 3B

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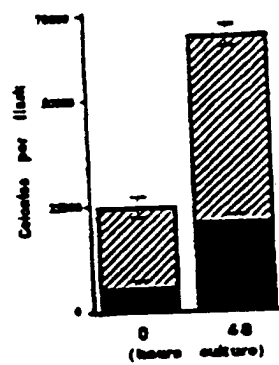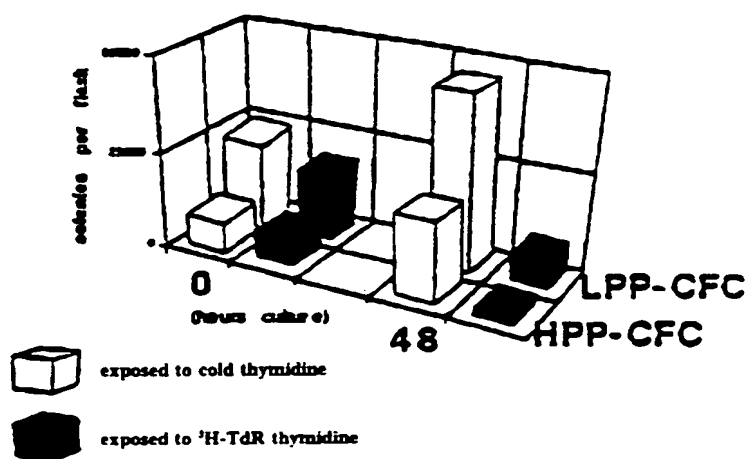
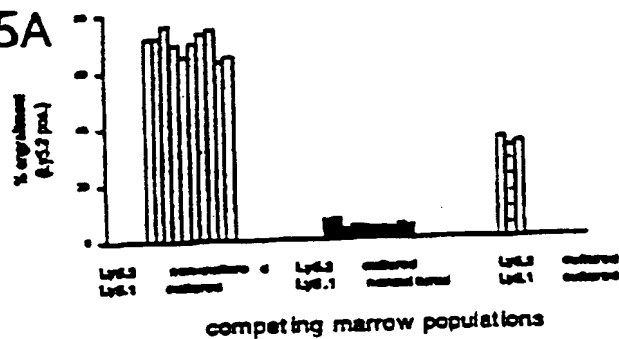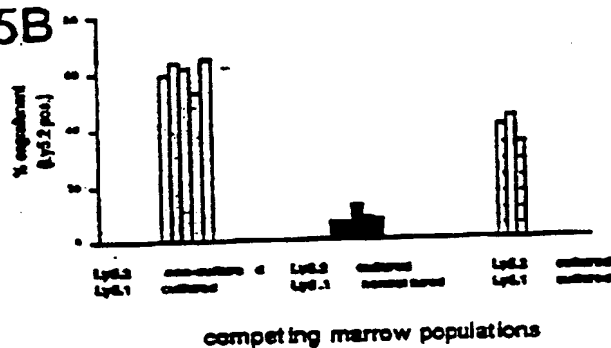*Fig.* 4A   C57Bl/6   (Ly5.2)

Fig. 4B   B6.SJL   (Ly5.1)

□ exposed to cold thymidine

■ exposed to ³H-TdR thymidine

Fig 5A

12 weeks

Recipient strain:
Ly5.1
(B6.SJL)

competing marrow populations
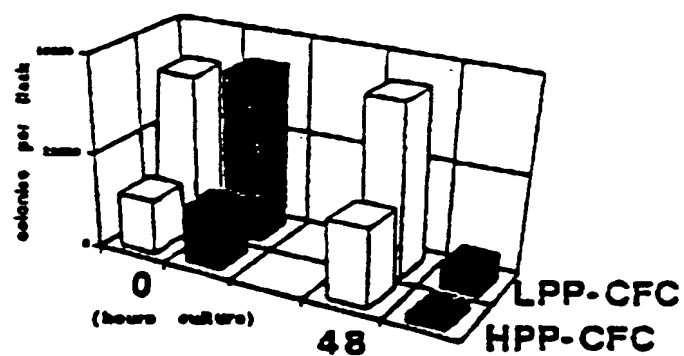
Fig. 5B

12 weeks

Recipient strain:
Ly5.2
(C57 BL/6)

competing marrow populations

Fig. 5C

22 weeks

Recipient strain:
Ly5.1
(B6.SJL)

competing marrow populations